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THE USE OF A DARK ADAPTATION TECHNIQUE (BIOPHOTOMETER) IN THE MEASUREMENT OF VITAMIN A DEFICIENCY IN CHILDREN [1]

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INTRODUCTION

Evidence in recent literature indicates an increasing appreciation of the need, in practical nutrition work, for tests for the measurement of specific nutritional deficiencies. From the public health viewpoint the need for such tests is obvious, since administrative and remedial programs directed toward the eradication of nutritional defects must be predicated on definitive information concerning the nature, extent, and distribution of such deficiencies in the general population.

Among suggested methods, the quantitative dark adaptation test for measuring vitamin A deficiency constitutes one of the most promising developments in this field. Certain earlier work on this test was based on observations made with the Birch-Hirschfeld photometer, as described by Jeans and Zentmire (*1*) in 1934. In a survey of Iowa school children, Jeans and Zentmire (*2*) found that between 26 and 79 percent of the children examined had definitely subnormal dark adaptation measurements and therefore showed evidence of vitamin A deficiency. During the course of his studies I. O. Park (*3*, *4*, *5*) found a high prevalence of deficiency, 83 percent being given for one mixed group of adults and orphan Indian children in Oklahoma. Jeghers (*6*) found a 34 percent deficiency among supposedly healthy adults. Snelling (*7*), however, concluded that the Birch-Hirschfeld photometer was unsatisfactory for measuring small differences in dark adaptation.

The shortcomings of the Birch-Hirschfeld photometer have stimulated the development of a new instrument, the "biophotometer." [2] In a recent report on the development of the biophotometer test, Jeans, Blanchard, and Zentmire (*8*) essentially confirmed their previous findings and concluded that between 16 and 69 percent [3] of

[1] From the Division of Public Health Methods, National Institute of Health.

[2] Name given to instrument by the manufacturers, Frober-Faybor Co., of Cleveland, Ohio.

[3] Corrected from 26 and 79 percent according to this later work (*8*), in which it is reported: "When a larger and perhaps more reasonable degree of experimental error is allowed, the proportion of children with definitely subnormal adaptation becomes 10 percent less in each instance."

the previously surveyed children showed evidence of vitamin A deficiency. As a result of these reports and their obvious public health implications, investigations for the purpose of obtaining additional data on the use of the biophotometer test in general school health work have been undertaken. The present paper deals with an evaluation of certain aspects of the biophotometer test as a survey technique, especially in children.

THE BIOPHOTOMETER TECHNIQUE

The measurement of dark adaptation of the human eye, while relatively new as a practical health test, has been under investigation for a number of years. As a result of this work, techniques for the measurement of adaptation have been developed, and a not insignificant body of knowledge has accumulated regarding the mechanism of adaptation. It may be expected that this knowledge, which includes a considerable amount of observational and theoretical data, would be pertinent to the present problem involving the study of dark adaptation of the human eye. It is evident, however, that the dark adaptation measurements used for the vitamin A studies are largely empirical. Also, it is not yet possible to fit the results of more recent studies into preexisting facts and theories regarding the physiology of adaptation. While it is of great importance that these somewhat separate lines of investigation be correlated as soon as possible, the practical importance of the vitamin A studies undoubtedly justified further investigation in spite of a considerable number of apparent discrepancies between the newer studies and generally accepted facts regarding adaptation. In line with this view, the present paper will attempt no clarification of these apparent discrepancies. An attempt will be made only to evaluate briefly and in somewhat general terms the biophotometer test as an *empirical test.*

In brief, the biophotometer technique may be described as follows: The subject is placed in absolute darkness for a prescribed interval of time, at the end of which a preliminary measurement is made of the threshold or lowest intensity of light that is just perceptible to him. The subject is then asked to look directly at a bright light in order to "light-adapt" or "bleach" the eyes. After a short period of exposure to this light the subject is again placed in darkness, and, at stated intervals, measurements are made to determine the thresholds of light intensities.

The test is a simple one, and the general qualitative nature of the results is a matter of common knowledge. It is a matter of everyday experience that a relatively bright light cannot be perceived immediately after looking at a much brighter one and that the longer one remains in darkness, within limits, the less intense a light needs to be in order to be perceptible. In making quantitative measurements of

this physiological process, the biophotometer is used to obtain uniform conditions of exposure of the eyes to the bright light and for measuring the thresholds of light.

In evaluating the test merely as a test, it becomes necessary to consider the details of a number of steps in the experimental procedure. The first of these concerns the "bleaching" step. In the technique under discussion this consists of instructing the subject to look steadily, with both eyes open, for a period of 3 minutes, at a brightly illuminated ground glass screen within the biophotometer. A subject may or may not follow these simple instructions completely, and the operator has no definite way of knowing just how precisely the standard conditions of the test are being followed. With cooperative subjects, particularly adults, this part of the test probably is reasonably well controlled. Without the operator's knowledge, however, a subject may look at the black surfaces of the sides of the biophotometer or may even close the eyes during all or part of the period that he is instructed to gaze at the illuminated screen. Three minutes may seem a long time to some individuals, especially to children, and there can be little doubt that a considerable amount of variability in the results is possible and probable because of lack of rigorous control over this bleaching procedure. The present biophotometer test does not provide for the control of this variation, and it is difficult or perhaps impossible to make a quantitative evaluation of the amount or importance of this source of variability in routine use of the present instrument.

The second step in the test that needs particular attention concerns the determination of the thresholds of light perceived by the subjects under test. According to the standardized procedure adopted for the vitamin test, four threshold determinations are made, the first 20 to 30 seconds after the bright or bleaching light is turned out and the others at approximately 3-minute intervals, the last being made 10 minutes after bleaching. The steps in a threshold determination are as follows: The subject is asked to look at a black screen in the center of which is a quincunx of small circular openings which appear as disks of light. The intensity of the illumination of the five disks in the quincunx varies in two ways. First, a wedge placed between a source of light and the five openings in the black screen changes the intensity of light from right to left in such a way that the two right-hand disks appear the least bright, the center disk as of intermediate brightness, while the left-hand disks appear the brightest. The second mechanism for varying the illumination of the quincunx consists of an electric light connected in series with a rheostat which permits the variation of light intensity by turning the dial of the rheostat. The dial of this rheostat is marked off in arbitrary units from 0 to 100, and a table supplied with each biophotometer furnishes

data for converting each dial reading to a calibrated intensity of light, expressed in millifoot candles (mfc.) of the illumination of the center disk in the quincunx.

Since the essential results of the biophotometer test must be expressed in terms of these readings, it is pertinent to examine carefully the method of recording them. The first point for consideration in this connection concerns the arbitrary dial units, which consist of a simple arithmetic scale from 0 to 100. This scale actually represents an arbitrary logarithmic scaling of the light intensities as measured in millifoot candles. In actual practice, therefore, the thresholds of light intensities are observed on a scale which is a logarithmic scale of millifoot candles of light intensities. For the purposes of final uses of the readings, however, it is customary to convert the logarithmic values to an arithmetic scale. This procedure, that is, the taking of measurements on a logarithmic scale and the use of the measurements on an arithmetic scale, introduces a question of mensuration which has considerable import in the final interpretation of results. An example will illustrate this point. In one biophotometer used in the work an arbitrary dial reading of 5 is equivalent to a mfc. reading of 4.7, and a dial reading of 6 is equivalent to 4.4 mfc. The difference between these two dial readings equals, therefore, one dial unit or 0.3 mfc. On the same biophotometer a dial reading of 80 is equivalent to 0.0035 mfc. and a dial reading of 81 is equivalent to 0.0032 mfc. The difference between these two successive dial readings is, therefore, one dial unit,[4] but at this light intensity this equals only 0.0003 mfc., or about one-thousandth the light intensity difference at the higher range of the scale. Thus, equal changes on the dial scale represent very different changes on the mfc. scale and serve to raise the question as to what scale measures most precisely the physiological function desired in the test.

Obviously, it may be difficult to determine the most effective physiological scale; and in line with the expressly stated purpose of this paper, no attempt will be made to consider this question in detail. On the other hand, the necessary consequences of the method adopted for recording the biophotometer readings must be considered. Consideration of this matter is forced when an attempt is made to determine the reliability and consistency of biophotometer test readings. A simple example will illustrate this point. In making preliminary tests with adults, it was found that changes of one or two arbitrary dial units are usually just perceptible to the eye of a presumably normal person when the threshold is at the level of about 25 dial units. In this range of threshold values, it is apparent that the eye is distinguishing between mfc. values of approximately 0.63 and 0.76. When,

[4] It may be important to note that no attempt can be made to read intensity values to proportionate parts of a single dial unit. The practical reason for this will be brought out later.

however, the threshold values are near the level of 80 dial units, it becomes apparent that the eye is capable of distinguishing only the difference between 3 to 4 dial unit changes, or approximately the difference between 0.0035 and 0.0052 mfc.

These observations indicate that measurement of the physiological process is approximately accurate from one to two arbitrary dial units (logarithms of mfc.) at one end of the range of the physiological process and about four dial units at the other end of the range. On the mfc. scale, measurement of the physiological process, on the other hand, is accurate to 0.13 mfc. at one end of the range and 0.0017 mfc. at the other end. In terms of logarithms of mfc., therefore, measurements are about twice as accurate at one end as at the other end of the scale, while in terms of actual mfc., measurements are roughly 100 times as accurate at one end as at the other end of the scale. It would be advantageous if the same scale units had the same meaning with respect to the precision of measurements over the entire range of experience of measurements. Obviously, neither the dial units (logarithms of mfc.) nor the mfc. units themselves fill this need, and it is apparent that a study of the accuracy or precision of the threshold measurements cannot be made on either of these scale units without considering the level of the threshold intensities. Since it seems necessary for present practical purposes to select one of these scales, the advantages of the logarithms of the mfc. definitely make this scale preferable.

The third characteristic of the biophotometer test that requires consideration also deals with the determination of the thresholds of light perceived. While being tested the subject is shown the quincunx of light spots and is asked to tell the operator at what light intensity the center spot of the quincunx is just visible. According to the method proposed for the test, the operator reduces the light intensity of the quincunx until the subject says that only the two left hand spots are visible and then increases the light intensity until the subject says he just perceives the center spot. The reading on the biophotometer scale at this point is then recorded as the threshold for that particular trial. The important fact to be emphasized at this point is that the basic reading of the test depends upon a subjective judgment by the person being tested.

Two extremely important considerations arise in connection with this subjective characteristic of the test. According to the present design of the biophotometer, the operator of the test has no way of knowing whether or not the subject actually "sees" the critical spot of light. Most subjects will obviously give a truthful answer regarding the perception of the light, but the operator has no way of knowing what criteria the subject employs in perceiving the threshold, and considerable variation may arise because of the difficulties encountered,

especially by children, in describing the critical point of the test. Some individuals will report that they perceive the light when it is very indistinct indeed and even when there may be some doubt in their minds that they actually see it. Other individuals—and ordinarily the operator would be quite unable to say which individuals these are—may report seeing the test light only if it is very distinctly visible. This last difficulty is not unique for the biophotometer test; it is basic in the determination of any sensory threshold.

From one point of view, however, the uses made of the adaptation test for vitamin A nutrition are different from the uses most often made of a sensory threshold measurement. Determinations of thresholds of auditory acuity, for example, are made usually for the purpose of determining effective hearing; and if one individual requires a more distinct sound than another in order to report a sensory perception, the effective hearing of the former probably is actually less than that of the latter. The determination of the light threshold in the biophotometer test, on the other hand, is made only for its indirect value in diagnosing the state of vitamin A nutrition. For example, suppose an individual consistently, over a long series of tests, requires a rather bright light for the threshold measurement. Is this due to a low or subnormal adaptation, or does it represent, to a certain extent, a different interpretation of what the threshold means? Also, is a person giving such results on the test, who is on a high and adequate vitamin A diet, "refractory" to vitamin therapy, or is he, in some degree, showing a different interpretation of threshold values? Such questions clearly indicate the complexity of the biophotometer tests and exemplify the caution that is necessary in interpreting the results of the adaptation measurement as showing the status of vitamin A nutrition.

The biophotometer test, however, resembles other sensory threshold tests in its subjective character. The significant implications of this similarity have not been emphasized in reports on the biophotometer, since very little attention has been given to the evaluation of two important characteristics of such subjective tests: First, the high individual variability; and, second, the "practice" or "learning" factor which has been found in some other types of threshold measurements. The accumulated experience of a large amount of physiological and psychological work on other threshold tests indicates the importance of analyzing the amount and sources of variations before the measurement of an individual can be considered as reliably characteristic of that individual. The same work also indicates the tremendous importance of repeated tests on the same individual in order to establish the reliability of an individual's threshold measurement.

The above considerations are sufficient, it would seem, to indicate certain basic characteristics of the biophotometer test as a test and to show the difficulties inherent in interpretations of the test as a method of measuring, even empirically, vitamin A nutrition.

INTERPRETATION OF BIOPHOTOMETER TESTS IN THE MEASUREMENT OF VITAMIN A NUTRITION

According to the report of Jeans and coworkers (*8*), a classification of individuals with respect to vitamin A nutrition can be based on readings of the thresholds of light intensities determined with the biophotometer. These workers indicate that the primary basis of this classification depends on the threshold found at the "first", and sometimes at the "last", recovery reading. The "first" recovery reading is made in the interval 20 to 30 seconds after light-adapting, or "bleaching", the eyes; the "last" recovery reading is made 10 minutes after light-adapting the eyes. While it is indicated further that other threshold readings are considered in making a final diagnosis of the state of vitamin A nutrition, the use of such other readings is not clearly explained. The classification of individuals on the basis of "first" and "last" recovery reading into three groups, "normal", "borderline", and "subnormal", is shown in table 1.

TABLE 1.—*Millifoot candle (mfc.) values used for classifying biophotometer tests*

Classification	"First recovery" (20–30 seconds)	"Last recovery" (10 minutes)
"Normal"	0.6 mfc. or below	0.05 mfc. or below.
"Borderline"	0.6–1.0 mfc.	0.05–0.10 mfc. (implied).
"Subnormal"	Above 1.0 mfc.	Above 0.10 mfc. (implied).

The report of Jeans and coworkers is interpreted as indicating that an individual is classified as "borderline" or "subnormal" if either the "first" or the "last" recovery reading deviates from "normal." For example, if either the first or last reading falls into the range designated as "subnormal", the individual is classified in the "subnormal" group; if either the first or last reading falls into the "borderline" class but neither falls into the "subnormal" class, the individual is classified in the "borderline" group, and if neither reading falls below the ranges of the "normal" class, the individual is classified as "normal."

THE PRESENT STUDY

Data available for this report include, among other observations, the results of biophotometer tests on 585 children who were in attendance in the third to the eighth grades of the elementary schools in Maryland and the District of Columbia during the winter and

spring of 1936–37. Of these children, 177 were from a consolidated school in a rural mountainous area in western Maryland, 279 were from public schools in a small city in central Maryland, and 129 were urban children in the District of Columbia. Both Maryland groups were composed principally of children from families of low economic level, and the urban group was made up of children from institutional homes. The selection of children for the test was made merely on the basis of presence in the school at the time of the examination. Since it was apparent that satisfactory testing cannot be accomplished generally in children below the second grade, the work was begun in the third grade and continued in successively higher grades as time permitted. The method of making the tests followed in detail the technique described by Jeans, Blanchard, and Zentmire (*8*).

For the purpose of comparing the results obtained in this survey with those obtained in Iowa, the classification of tests proposed by Jeans and coworkers is used for reporting the data shown in table 2. In general, the results of the surveys in the midwestern and eastern localities are roughly comparable with respect to the proportion of adaptation tests classified as "subnormal." The proportions of individuals falling into the "borderline" class seem to be definitely higher in the eastern group, while the proportions falling into the "normal" group are generally lower. These results would appear to indicate that the differences between the midwestern and eastern localities, and between the subgroups within the two localities, are greater than are likely to arise fortuitously.

TABLE 2.—*Comparison of results of biophotometer tests in school children*

Classification	Maryland and District of Columbia, 585 children			Iowa,[1] 404 children		
	Rural	Small city	Urban	Rural	Village	Urban
	PERCENT OF CHILDREN					
"Normal"	33	11	23	64	37	34–11
"Borderline"	59	32	35	20	20	20
"Subnormal"	8	57	42	16	43	[2] 46–69
Total	100	100	100	100	100	100
	NUMBER OF CHILDREN TESTED					
	177	279	129	100	102	202

[1] From the survey report of Jeans and Zentmire (*2*), but corrected as indicated by Jeans and coworkers (*8*) for conversion into biophotometer results. (See footnote 3, p. 1403.)
[2] Upper economic level 46 percent; middle 53 percent; and lower 69 percent.

A number of points may be raised as possible explanations of the differences. The first of these considerations involves a quantitative study of the errors of measurement of the biophotometer test.

Obviously, a sufficiently complete analysis of errors of measurement of the complex series of measurements that make up the complete biophotometer test would alone require a very extensive investigation. Neither our own work nor that of others reported in the literature is sufficient, as yet, for such an analysis, so that any present evaluation of the precision of the measurements in the interpretation of results must be based on a few general and somewhat unsatisfactory considerations.

As has already been mentioned, preliminary observations with the biophotometer indicate that, in the middle range of light intensity measurements, most individuals are usually able to define their thresholds within a range of one to two arbitrary dial units, but in the range of the lowest light intensity, they are usually able to define their thresholds within about four arbitrary dial units. What do these simple observations mean with respect to the precision of classification of individuals into the groupings, "normal", "borderline", and "subnormal"? An answer to this single question alone requires an analysis and evaluation of readings that present difficult and time-consuming statistical and experimental work. It must be sufficient at this point, therefore, to indicate that in many cases the classification of an individual into the "subnormal" rather than the "borderline" class or into the "borderline" rather than the "normal" class depends upon a subjective judgment of the threshold of light. This, in turn, means the differentiation of one dial unit of intensity in a situation where it is impossible for the ordinary subject to differentiate between two dial units of light intensity.

Another significant part of the evaluation of the reliability of the measurements, and one which at this time can be answered only in very general qualitative terms, concerns the variation in tests made in immediate succession. In their report on the development of the biophotometer, Jeans and coworkers have indicated that the experimental error is not great enough to interfere with proper interpretation of results. Although very little quantitative data are given, it appears that a variation of 4 dial units, or 0.2 mfc., may occur in the "first" recovery reading during successive tests of a child classified as "normal" according to the proposed criteria. Our preliminary work indicates that a variation of approximately 4 dial units, or 0.2 mfc., occurs very frequently in the readings of a cooperative adult whose threshold falls in the "normal" range, and that a variation of approximately 4 dial units or 0.3 mfc. occurs frequently in successive readings of an adult whose threshold falls in the "borderline" range. Part of this variation is, of course, the "error of measurement" inherent in a single biophotometer test, but a significant part is probably due to physiological variation of the individual. Our preliminary observations on cooperative subjects suggested that the test-to-test

variation in children may well be as much as 5 or 6 dial units (0.4 or 0.5 mfc.), a difference sufficient to cause changes of classification from "subnormal" to "borderline", or even from "subnormal" to "normal" in a succession of only 3 or 4 tests.

In their recent report Jeans, Blanchard, and Zentmire (*8*) have indicated that a single biophotometer test is probably not sufficient to establish the fact of deficiency in an individual child. In order to test further the significance of this suggestion, 247 of the children [3] previously surveyed were retested within a few days after the initial test. The second testing gave results which, when classified according to the specified groupings, produced a new arrangement of the children. The classification of the data for the first and second tests is shown in table 3. It may be noted that the percentage of "subnormal" tests is decreased from 56.3 to 35.6 percent simply by testing a second time. This marked change on the second test in the proportion of children included in the various groups suggested the value of more successive tests in order to determine whether or not still further changes would occur. Accordingly, 100 subjects, among whom were included practically all of those with decidedly "subnormal" readings, were given a sequence of four tests. These were usually made twice a week over a 2-week period. The classifications of results from the first and fourth tests are shown in table 4.

TABLE 3.—*Classification of children on the basis of first and second biophotometer tests (247 children)*

Classification	Number of children		Percent of children		Proportionate change, first to second test
	First test	Second test	First test	Second test	
"Normal"	26	50	10.5	20.3	93 percent increase.
"Borderline"	82	109	33.2	44.1	33 percent increase.
"Subnormal"	139	88	56.3	35.6	37 percent decrease.

TABLE 4.—*Classification of children on basis of first and fourth biophotometer tests (100 children)*

Classification	Number of children		Proportionate change, first to fourth test
	First test	Fourth test	
"Normal"	9	26	189 percent increase.
"Borderline"	32	45	41 percent increase.
"Subnormal"	59	29	51 percent decrease.

It is apparent from these figures that retesting causes a very significant improvement in the readings, so that, after a sequence of four tests, the proportion of children falling into the group designated as "subnormal" is only about one-half that obtained on the basis of a single test.

[3] In the selection of these 247 children an attempt was made to include as many as possible of those who gave "subnormal" readings on the first test.

These significant changes suggest the presence of what may be termed the "practice" or "learning" factor, which is frequently encountered in threshhold measurements. In order to obtain more specific data on this question a group of 41 children, consisting principally of those whose initial tests were classified in the "subnormal" or "borderline" groups, were given a sequence of eight tests, extending over a period of approximately 5 weeks. A detailed analysis of the results of the eight sequence testings is given in tables 5 and 6; and, in order to present the changes more clearly, the results of the "first" and "last" recovery readings are given separately. On the left-hand side of table 5 is given frequency distribution, for the "first" recovery reading on each of the eight successive tests, of the number of children whose readings fell at each dial and mfc. level provided for on the biophotometer scale. On the right-hand side of this table are given the percentage distributions of the results accumulated from the highest to the lowest mfc. values. Precisely similar data are given in table 6 for the "last" recovery reading, which Jeans and coworkers regard as a secondary criterion.

TABLE 5.—*Frequency distributions of "first recovery" biophotometer readings, 8 successive tests*

Biophotometer readings			Actual frequency Number of children								Accumulated frequency Percent of children							
			Test								Test							
Dial units		mfc.[1]	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth
	8	3.50	1								2							
	9	3.15									2							
	10	2.90	1							1	5							2
	11	2.60			1						5		2					2
	12	2.40		2			1				5	5	2		3			2
	13	2.16	2							1	10	5	2		3			5
"Subnormal"	14	1.95			1		1				10	5	5		5			5
	15	1.80	6	1	2	2	1			2	24	8	10	5	8			10
	16	1.60	2		1		1	1	2		29	8	12	5	10	3	5	10
	17	1.48	2	2			1			1	34	13	12	5	13	3	5	12
	18	1.36	1	4	3	4	1	2	1		37	23	20	15	15	8	8	12
	19	1.22	2	1	2		1		1		41	25	24	15	18	8	11	12
	20	1.10	5	7	5	7	2	5	3	2	54	43	37	32	23	20	18	17
	21	1.00	7	4	4	1	4	1	3	2	71	53	46	34	33	23	26	22
	22	.92	2	4	2	5	5	8	4		76	63	51	46	46	43	37	22
"Borderline"	23	.84	2	5	5	4	2	3	3	3	80	75	63	56	51	50	45	29
	24	.76		2	2	4	5	3	3	4	80	80	68	66	64	58	53	39
	25	.69	2	4	3	3	2	5	4	4	85	90	76	73	69	70	63	49
	26	.63	2	3	1	3	5	1	2	4	90	98	78	80	82	73	68	59
	27	.58	1		4	2	2	2	4	3	93	98	88	85	87	78	79	66
	28	.52	1		3	3		2	1		95	98	95	93	87	83	82	66
	29	.47			1	2		2	1	3	95	98	98	98	87	88	84	73
	30	.44	2	1	1	1	2	3	2	3	100	100	100	100	92	95	89	80
	31	.38					2		1	2					97	95	92	85
"Normal"	32	.35							1	2					97	95	95	90
	33	.315						1	2						97	98	100	90
	34	.290						1		1					97	100		93
	35	.260								1					97			95
	36	.240								1					97			98
	37	.216					1								100			98
	38	.195								1								100
Total			41	40	41	41	39	40	38	41								

[1] mfc.=millifoot candles.

TABLE 6.—*Frequency distributions of "last recovery" biophotometer readings, 8 successive tests*

Biophotometer readings			Actual frequency Number of children								Accumulated frequency Percent of children							
			Test								Test							
Dial units		mfc.[1]	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth
	28	0.52	1	----	----	----	----	----	----	----	3	----	----	----	----	----	----	----
	29	.47	----	----	----	----	----	----	----	----	3	----	----	----	----	----	----	----
	30	.44	----	----	----	----	----	----	----	----	3	----	----	----	----	----	----	----
	31	.38	----	----	----	----	----	----	----	----	3	----	----	----	----	----	----	----
	32	.35	----	----	----	----	----	----	----	----	3	----	----	----	----	----	----	----
	33	.315	----	----	----	1	----	----	----	----	3	----	----	3	----	----	----	----
	34	.290	----	----	----	----	----	----	----	----	3	----	----	3	----	----	----	----
	35	.260	----	----	----	----	1	----	----	1	3	----	----	3	3	----	----	2
"Subnormal"	36	.240	----	----	----	----	----	----	----	----	3	----	----	3	3	----	----	2
	37	.216	----	----	----	----	----	----	----	----	3	----	----	3	3	----	----	2
	38	.195	----	----	----	----	----	----	1	----	3	----	----	3	3	----	3	2
	39	.180	----	----	----	----	----	----	1	----	3	----	----	3	3	----	5	2
	40	.160	3	1	1	1	----	1	----	1	10	2	2	5	3	2	5	5
	41	.148	----	----	----	----	1	----	----	----	10	2	2	5	5	2	5	5
	42	.136	----	----	----	----	----	----	----	1	10	2	2	5	5	2	5	7
	43	.122	1	----	----	1	----	----	----	----	13	2	2	8	5	2	5	7
	44	.110	----	3	1	----	----	----	----	----	13	10	5	8	5	2	5	7
	45	.100	1	1	1	1	----	2	----	1	15	12	7	10	5	7	5	10
	46	.092	----	1	1	----	----	2	----	----	15	15	10	10	5	12	5	10
	47	.084	1	----	1	----	1	----	----	1	18	15	12	10	8	12	5	12
"Borderline"	48	.076	1	----	----	----	----	----	----	1	20	15	12	10	8	12	5	15
	49	.069	----	----	1	1	1	----	----	1	20	15	15	13	11	12	5	17
	50	.063	3	1	2	2	----	2	2	1	28	17	20	18	11	17	11	20
	51	.058	2	1	----	1	----	----	1	----	33	20	20	20	11	17	13	20
	52	.052	2	3	2	2	----	----	----	----	38	27	24	25	11	17	13	20
	53	.047	----	3	----	3	----	----	3	2	38	34	24	33	11	17	21	24
	54	.044	1	2	3	1	----	1	1	----	40	39	32	35	11	20	24	24
	55	.038	5	3	7	1	----	1	1	2	53	46	49	38	11	22	26	29
	56	.035	----	2	2	----	----	4	2	----	53	51	54	38	11	32	32	29
	57	.0315	2	3	2	2	4	1	2	----	58	58	59	43	21	34	37	29
	58	.0290	1	----	3	1	4	4	----	1	60	58	66	45	32	44	37	32
	59	.0260	----	----	2	1	2	----	4	3	60	58	71	48	37	44	47	39
	60	.0240	2	5	2	1	2	3	----	1	65	71	76	50	42	51	47	41
	61	.0216	3	----	1	3	2	2	----	2	73	71	78	58	47	56	47	46
	62	.0195	2	2	1	1	3	1	1	4	78	76	80	60	55	59	50	56
	63	.0180	4	2	----	----	1	1	----	2	88	80	80	60	58	61	50	61
	64	.0160	----	----	----	3	----	3	2	2	88	80	80	68	58	68	55	66
	65	.0148	----	2	----	2	3	4	2	2	88	85	80	73	66	78	61	71
	66	.0136	2	1	----	3	3	1	1	2	93	88	80	80	74	80	63	76
	67	.0122	1	2	2	2	1	----	1	----	95	93	85	85	76	80	66	76
"Normal"	68	.0110	----	1	----	2	3	3	1	2	95	95	85	90	84	88	68	80
	69	.0100	----	----	2	1	----	1	2	2	95	95	90	93	84	90	74	85
	70	.0092	1	1	2	----	3	2	4	1	98	98	95	93	92	95	84	88
	71	.0084	----	----	2	----	----	1	----	2	98	98	100	93	92	98	84	93
	72	.0076	----	----	----	1	----	----	2	----	98	98	----	95	92	98	89	93
	73	.0069	----	----	----	----	----	----	----	1	98	98	----	95	92	98	89	95
	74	.0063	----	----	----	----	----	1	----	----	98	98	----	95	92	100	89	95
	75	.0058	1	1	----	1	1	----	3	1	100	100	----	98	95	----	97	98
	76	.0052	----	----	----	----	1	----	----	----	----	----	----	98	97	----	97	98
	77	.0047	----	----	----	----	----	----	----	----	----	----	----	98	97	----	97	98
	78	.0044	----	----	----	1	----	----	----	----	----	----	----	100	97	----	97	98
	79	.0038	----	----	----	----	----	----	----	----	----	----	----	----	97	----	97	98
	80	.0035	----	----	----	----	1	----	----	----	----	----	----	----	100	----	97	98
	81	.0032	----	----	----	----	----	----	----	----	----	----	----	----	----	----	97	98
	82	.0029	----	----	----	----	----	----	----	----	----	----	----	----	----	----	97	98
	83	.0026	----	----	----	----	----	----	----	----	----	----	----	----	----	----	97	98
	84	.0024	----	----	----	----	----	----	1	1	----	----	----	----	----	----	100	100
Total			40	41	41	40	38	41	38	41								

[1] mfc. = millifoot candles.

An example may facilitate the reading of these tables. Thus on the first test (table 5, left-hand section) one child gave a "first" recovery reading of 8 dial units, or 3.5 mfc.; another child gave a reading of 2.90 mfc.; two children gave readings of 2.16 mfc.; and so on. On the

right-hand side of this table, in the column marked "first test", the frequency of cases is accumulated in percentages. For example, 2 percent of the children gave readings of 3.50 mfc. or more; 5 percent of the children gave readings of 2.90 mfc. or more; and 10 percent of the children gave readings of 2.16 mfc. or more. The accumulation of the frequency percentages permits, therefore, a statement of the percentage of cases which fall on or above any specified mfc. value. Thus, the percentage of children who are above the 1.0 mfc. value, one of the criteria specified as marking the lower limit of the class designated as "subnormal", is 54 on the first test. It may be observed that these children, classified as having readings above 1 mfc., are automatically, by the proposed classification, designated as giving "subnormal" dark adaptation tests. According to the proposed interpretation of the tests, therefore, these children are deficient in vitamin A. On the second test 43 percent of the children fell into this class. In each succeeding test the proportion of children designated in the "subnormal" class decreases as shown in the table and finally, on the eighth and last test, only 17 percent of the children are placed in this group. Thus, after eight successive tests the proportion of children falling into the class designated as "subnormal" is reduced from 54 percent to 17 percent. A consideration of the proportion of cases classified as "normal" also shows the significant effect of "practice" or "learning." Thus, on the first test only six children were classified as "normal", but on the eighth test 21, or more than 50 percent, were so classified.

It is of considerable interest to find, also, that two of the seven children comprising the 17 percent in the designated "subnormal" class on the eighth test were in the "borderline" group in the first test. Thus it is seen that, although the general trend of measurements is toward improvement, the marked variations which are observed result in very significant rearrangement of children in the successive classifications. This illustrates the variability inherent in these tests, a consideration of which will receive full treatment in a subsequent paper. However, the data given here show that, in spite of the observed variabilities, very definite improvement occurs, on the average, in successive tests.

During the preceding analysis of the significance of the learning factor, the standards proposed by Jeans and coworkers (*cf.*, table 1) were used for the classification of subjects as "normal", "borderline", and "subnormal", with respect to dark adaptation. Since "subnormal" dark adaptation is taken to indicate vitamin A deficiency, it is of importance to inquire into the validity of these critical values in relation to this interpretation. The most direct way of demonstrating a relationship between the dark adaptation test and vitamin A would appear to be through feeding the vitamin to subjects with "subnormal" readings and observing any changes. Feeding experiments of

this kind were reported by Jeans and Zentmire *(2)* in their study with the Birch-Hirschfeld photometer. Although the great majority of the children were found to be normal when examined after the vitamin administration, the Iowa workers commented upon a few subjects who did not give normal readings despite the absence of any demonstrable ophthalmological or other abnormality. These few exceptions indicate either that the vitamin A ingested by these children was not utilized or that a "subnormal" dark adaptation test does not necessarily indicate vitamin A deficiency.

In the present survey the actual relationship between the biophotometer reading levels and vitamin A deficiency was investigated by means of a supplementary feeding experiment carried out during the months of February, March, and April 1937. The complete details of the experiment will be reported at a later date. At this point, however, it must be mentioned that of 25 children who gave rather consistent "borderline" or "subnormal" readings before the feeding was begun, 16 still did not give normal readings at the end of the feeding period of 6 to 8 weeks. During this time the dosage of vitamin A, given in the form of halibut liver oil capsules, was 18,000 International Units per day, which is equivalent to more than 8 teaspoonfuls of U. S. P. cod liver oil daily and far in excess of a child's estimated requirements. The total vitamin A intake of each child was 756,000 units during the 6-week period, and 1,008,000 units during the 8-week period. When the experiment was concluded, two of the children gave readings of 1.1 mfc. on the "first" recovery reading. This value is slightly within the range of "subnormal" dark adaptation ("subnormal" values are those above 1.0 mfc., cf. table 1), according to the standards of Jeans and coworkers. The other 14 children gave "borderline" readings, that is, from 0.6 to 1.0 mfc. These results demonstrated that an appreciable proportion of the children failed to attain or maintain biophotometer readings within the so-called "normal" range despite the prolonged ingestion of ample vitamin A.

It is possible that the vitamin A ingested was not utilized, but it seems highly improbable that so many apparently healthy subjects, free from gross visual defects, would fail to use a significant amount of the excess administered. Since all of the refractory cases were those in which the departures from "normal" were in the "first" recovery reading alone and which were "normal" by the "last" recovery reading, it appears that the range of "normal" should be extended if the first recovery reading, which hitherto has been considered of primary importance, is to be used as a criterion. On the basis of the feeding experiment, in which 18,000 I. U. per day must be regarded as a more than adequate dosage, "normal" readings may be as high as 1.1 mfc. These results constitute strong evidence

against the validity of the standards of classification that have been proposed.

DISCUSSION

In consequence of the foregoing data on the marked improvement associated with the "learning" factor, the marked variability in successive tests, and the strong evidence against the validity of the critical standards themselves, it is obviously impossible to make any definite statement regarding the incidence of vitamin A deficiency in the Maryland and District of Columbia school groups on the basis of the biophotometer tests. The biophotometer surveys certainly provide no evidence for believing vitamin A deficiency to be prevalent among the groups of school children examined.

This study has been concerned only with an investigation of the biophotometer technique as an empirical test for vitamin A deficiency, without regard for theoretical considerations. However, it is interesting to point out in discussion that the biophotometer dark adaptation test is said to be based upon the measurement of the rate of regeneration of the retinal visual purple, which in turn is considered a measure of the state of vitamin A nutrition of the body. It may be mentioned that, according to present knowledge of the subject (*cf.* review by Hecht (*9*)), the instrument and the test are poorly designed for the purpose. Perhaps a more satisfactory apparatus for measuring the regeneration of visual purple might permit the development of a reliable and sensitive routine test for moderate vitamin A deficiency in children.

It must be emphasized again that this work has been directed toward an investigation of the biophotometer test as a survey technique for detecting subclinical vitamin A deficiency among supposedly normal school children. Perhaps the test may become more consistent and reliable when used in the clinic in conjunction with other examinations. With individual attention the subject may be given a sufficient number of preliminary tests to insure the reliability of the measurements, and the results might be correlated with determinations of vitamin A in blood (*10*). More data concerning the quantitative relationships between vitamin A nutrition and dark adaptation tests are needed. At the present time, however, the biophotometer test does not appear to be a reliable method for application to the routine survey of school children.

In a subsequent paper further data will be presented on the analysis of variability in readings and on the detailed results of feeding experiments.

SUMMARY AND CONCLUSIONS

The biophotometer dark adaptation test has been used in surveys of 585 elementary school children in Maryland and the District of

Columbia. Considerable improvement in readings, apparently due to a learning factor, resulted from the repeated testing of sample groups and demonstrated that little dependence could be placed upon survey results obtained from a single test of each child. In addition to the learning factor, significant variability occurred in successive tests. As a result of feeding experiments, evidence was presented against the validity of present standards for interpreting results in terms of vitamin A deficiency.

The uncertainties of the learning factor, variability, and doubtful standards made it impossible to state definitely the incidence of deficiency in the groups surveyed. However, the survey results gave no evidence for believing that vitamin A deficiency was prevalent among the school groups studied. The biophotometer test does not appear to constitute an accurate or reliable technique for detecting mild degrees of vitamin A deficiency in the routine survey of school children.

ACKNOWLEDGMENTS

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REFERENCES

(1) Jeans, P. C., and Zentmire, Z.: A clinical method for determining moderate degrees of vitamin A deficiency. J. Am. Med. Assoc., **102:** 892 (1934).
(2) Jeans, P. C., and Zentmire, Z.: The prevalence of vitamin A deficiency among Iowa children. J. Am. Med. Assoc., **106:** 996 (1936).
(3) Park, I. O.: Preliminary observations on vitamin A deficiency as shown by studies with the visual photometer. J. Oklahoma State Med. Assoc., **28:** 359 (1935).
(4) Park, I. O.: Further observations on vitamin A deficiency as shown by studies with the visual photometer and clinically. J. Oklahoma State Med. Assoc., **29:** 129 (1936).
(5) Park, I. O.: Significance of vitamin A deficiency. Am. J. Digest. Dis. and Nutr., **3:** 193 (1936).
(6) Jeghers, H.: Night blindness as a criterion of vitamin A deficiency: Review of the literature with preliminary observations of the degree and prevalence of vitamin A deficiency among adults in both health and disease. Ann. Int. Med., **10:** 1304 (1937).
(7) Snelling, C. E.: A study of the Birch-Hirschfeld photometric test for vitamin A deficiency. J. Pediatrics, **9:** 655 (1936).
(8) Jeans, P. C., Blanchard, E., and Zentmire, Z.: Dark adaptation and vitamin A: A new photometric technic. J. Am. Med. Assoc., **108:** 451 (1937).

(9) Hecht, S.: Rods, cones, and the chemical basis of vision. Physiol. Rev., 17: 239 (1937).
(10) Wolff, L. K., Banning, C., and Van Eekelen, M.: Nutrition of various groups of families in the Netherlands (showing the vitamin A and C content, and investigation of blood and urine for the presence of these two vitamins). League of Nations, Quarterly Bulletin of the Health Organization, 5: 566 (1936).

STUDIES ON CHRONIC BRUCELLOSIS

II. Description of Techniques for Specific Tests

By ALICE C. EVANS, *Senior Bacteriologist, National Institute of Health, United States Public Health Service*

The plan of the three field investigations carried on by the National Institute of Health to determine the prevalence of chronic brucellosis was outlined in the first paper of this series. Altogether somewhat more than a thousand patients were examined for evidence of *Brucella* infection. Three specific tests were used to aid in diagnosis—the agglutination, opson-ocytophagic, and intradermal reactions. The techniques used in carrying out the tests are described in this paper. The significance of data obtained will be discussed in another paper of this series.

THE AGGLUTINATION TEST

The field investigators separated the blood serum from the clot, added an equal quantity of sterile glycerine, and mailed the samples to the central laboratory. The serums as received were, therefore, in a dilution of 1:2. They were tested for agglutinins serially in final dilutions of 1 to 10, 20, 40, 80, 160, 320, 640, and 1,280. The dilutions were made as follows: In the first tube of the series was placed 0.4 cc of the serum mixture and 0.6 cc of saline solution. The next dilution was made by carrying 0.5 cc to a tube containing 0.5 cc of saline solution, and so on until the dilution reached 1:640 in the last tube. From the last tube of the series 0.5 cc of the diluted serum was removed to an agglutinating tube and kept for further possible use. The addition of 0.5 cc of antigen to each tube of the series brought the serum to the desired dilutions.

All serums were tested for specific agglutinins against two *Brucella* antigens, prepared with variety *abortus* No. 456 and variety *melitensis* No. 428, respectively. These two strains have been used for many years in this and other laboratories for the preparation of antigens for serological studies.

On account of the cross agglutination reactions between *Brucella* and *Bact. tularense* all serums were also tested for agglutination of *Bact. tularense* antigen. Those giving a positive reaction with the *tularense* antigen and also with the *Brucella* antigens were regarded as

having come from cases of tularaemia if the titer with the *tularense* antigen was the higher.

The *Brucella* cultures were grown in Blake bottles on beef infusion agar containing 1 percent glucose. Each bottle was inoculated with the entire growth from one agar slant suspended in about 1.5 cc of physiological saline solution. Incubation was at 37° C. for 2 days. After the incubation, 15 cc physiological saline solution containing 0.5 percent formalin was added to each bottle, and the growth was washed from the agar by rocking the bottle in the hands. The dense bacterial suspension was removed to centrifuge tubes, and after standing in the refrigerator a few days it was centrifuged, the clear supernatant fluid was discarded, and saline solution containing 0.5 percent formalin was added to bring the turbidity to 20,000 parts per million of the silica standard. As needed, this suspension was further diluted (39 parts of saline solution to 1 part of antigen) to prepare the agglutinating antigens of a density equivalent to 500 p. p. m. of the silica standard. The *tularense* antigen of a density equivalent to 500 p. p. m. of the silica standard was prepared according to the method described by Francis.

For the test, 0.5 cc of the diluted antigen was added to each of the series of tubes containing an equal quantity of diluted serum. The tubes were placed in a water bath at 37° C., and at the end of an hour they were examined. If agglutination had occurred in the 1:640 dilution, it indicated that the titer would be high. In that case the 0.5 cc of diluted serum which had been set aside was further diluted serially and antigen was added. In this additional series the highest final dilution of the serum was 1:20,400. The racks of tubes were then replaced in the water bath and incubated 1½ hours longer, then they were removed to the refrigerator, where they remained until the next morning, when the readings were made. Complete sedimentation of antigen with clear supernatant fluid was indicated by 4; supernatant turbidity as in control tubes containing 25, 50, or 75 percent of antigen was designated by 3, 2, or 1. Readings of 4 and 3 were interpreted as positive; readings of 2 and 1 were interpreted as slightly positive.

In the great majority of positive samples the agglutinin reactions with the *melitensis* and *abortus* antigens were in the same dilution or differed by only one step in the series. In the case of 16 serums (1.6 percent) there was a more marked difference in the titers as determined by the two antigens. For example, one serum reacted with the *abortus* antigen in the 1:80 dilution, but failed to react with the *melitensis* antigen. Another serum reacted with the *melitensis* antigen in the 1:40 dilution but failed to react with the *abortus* antigen.

THE OPSONO-CYTOPHAGIC TEST

As compared with Neufeld's bacteriotropin test, or Wright's opsonic test, the technique of the opsono-cytophagic test is less exacting because the leucocytes remain suspended in the sample of blood tested. It proved to be practical in the field; for, on the whole, all three workers obtained consistent results, although all occasionally sent slides too unsatisfactory for reading. Huddleson's technique was followed with certain modifications.

All glassware was clean and sterile.

The blood specimens were collected in 5 cc amounts in tubes containing 0.2 cc of a 20 percent solution of sterile sodium citrate in physiological saline solution. The test was carried out within 6 hours after collection. The specimens were shaken thoroughly before mixing with the bacterial suspension.

The bacterial suspension was freshly prepared from 48-hour glucose agar cultures of *Brucella melitensis* variety *abortus*, No. 456. The growth was taken up in sterile physiological saline solution of pH 7.0 and adjusted to a density equivalent to 600 p. p. m. of the silica standard.

The test was carried out in agglutination tubes. To 0.1 cc of the citrated blood was added 0.1 cc of bacterial suspension. After thorough mixing, the tubes were placed in a water bath at 37° C. for 30 minutes.[1] Directly after removing the tubes from the water bath, a small amount of the sedimented cells was removed with a finely drawn capillary pipette. A large drop of the cell suspension was placed near one end of a thoroughly cleaned and polished glass slide and a spread was made in the usual way by dragging the drop over the slide by means of another slide held at an angle, stopping about a half inch from the end of the slide. Then the top slide was dragged back, this time being held loosely. The best smears were obtained if the movement was "nervous", giving a wavy, uneven spread.

The slides were dried as quickly as possible under an electric fan in front of an electric heater.[1] They were then treated to dissolve the red cells by immersion for 3 or 4 minutes in a solution containing 1 percent acetic acid and 5 percent formalin in distilled water. They were then rinsed off, blotted gently on bibulous paper, and stained.

The slides were stained with Bordet-Gengou's carbol toluidin blue.[2] Slides stained for about 15 seconds show the deeply stained nuclei of the leucocytes and the deeply stained bacteria on a clear background.

[1] Due to lack of equipment, some of the field workers modified the technique by using an incubator instead of a water bath, and by drying slides without the use of an electric heater.

[2] Bordet-Gengou's carbol toluidin blue is made by dissolving 5 grams of toluidin blue in 100 cc of alcohol, 500 cc of distilled water, and 500 cc of 5-percent phenol. One part of this dye is diluted with two parts of distilled water for staining the smears.

In the technique described, Huddleson's method of carrying out the opsono-cytophagic test was modified in turbidity standard, in the preparation of the smears, in the staining of the slides, in making the readings, and in interpreting the results. A discussion of all these modifications follows.

Turbidity standard.—The silica turbidity standard, which was first adopted by the United States Geological Survey, and later by the American Public Health Association, is to be recommended for its simplicity and the readiness with which it may be prepared in any laboratory. Its preparation is described in every edition of *Standard Methods of Water Analysis.* In our laboratory a single standard preparation is used—a suspension of 300 parts per million of silica in an 8-cc homeopathic vial. This choice of standard was made because at the given density black letters of ordinary type are barely legible through it. At this degree of legibility comparisons may be made with the greatest accuracy. Sealed with a paraffin-covered cork stopper the standard may be kept indefinitely without deterioration. The one standard serves to prepare a suspension of any desired density. For example, to prepare a bacterial suspension of a density equivalent to 600 p. p. m. of the silica standard as used for the opsono-cytophagic test, the procedure is as follows:

Into an 8-cc homeopathic vial, 0.2 cc of the dense bacterial suspension is placed, and water is added until the density matches that of the standard. If, for example, it requires 3.8 cc of saline solution to bring 0.2 cc of the heavy bacterial suspension to the density of the 300 p. p. m. standard, then 3.6 cc of saline solution is added to 0.4 cc of the heavy bacterial suspension to bring it to a density equivalent to 600 p. p. m. of the silica standard. A *Brucella* suspension of this density contains approximately 5 billion organisms per cubic centimeter.

Preparation of the smears.—At the beginning of the investigations the slides received from the field sometimes had smears so thin that it was impossible to find enough leucocytes to make a satisfactory reading. In response to a request that smears of sufficient thickness for reading be spread over a larger area of the slide, the described technique for preparing the slides was devised by Dr. Royall M. Calder, who conducted the investigation in San Antonio, Texas. On some of the slides areas were found in which the smear was too thick, with the leucocytes in clumps. If the slide was properly prepared, however, areas with isolated leucocytes suitable for reading could readily be found.

Staining the slides.—The Hasting's stain, which is recommended by Huddleson, was found to be less satisfactory than Bordet-Gengou's carbol toluidin blue, which we had found most satisfactory in phagocytic studies made many years ago.

Reading the slides and interpreting results.—For the reading of slides and the interpretation of results we devised a method more simple than Huddleson's.

The wearisome task of counting bacteria ingested by leucocytes was omitted without loss of accuracy in interpretation. Indeed, the counting of ingested bacteria may be a source of error when the numbers are few, because it is not the number of bacteria that appear to be within the leucocyte which determines whether the reaction of the given cell is positive or negative. The significant point to be determined is whether the bacteria which appear to be within the leucocyte are more numerous than in an equivalent area of the surrounding field.

If two samples of blood are tested with bacterial suspensions of exactly the same density, and smears are prepared of exactly the same thickness, the number of bacteria in a field the size of a leucocyte may differ greatly on the two slides, depending on whether or not agglutination takes place. Negative chemotaxis does not occur. Hence, on one slide there may be an even distribution of bacteria, with some lying over the leucocytes and appearing to be ingested. On the other slide the bacteria may be agglutinated, with most of the inactive leucocytes standing in a clear field. Thus, if the presence of bacteria apparently within a leucocyte is regarded as signifying a positive reaction, many of the cells of the first slide may be erroneously recorded as positive, whereas that error would not be so likely to be made on the second slide. For this reason a rapid decision, made at a glance which takes in the surrounding field as well as the leucocyte, may result in more accurate judgment than the arduous counting of cells.

Twenty-five isolated polymorphonuclear leucocytes were examined, chosen from at least two separated areas. Leucocytes with no more bacteria than in a corresponding area of the surrounding field were recorded as negative. Those with more bacteria than in the surrounding field were recorded as positive; and if the leucocyte was filled with bacteria a circle was drawn around the plus sign. A leucocyte was regarded as "filled" if it contained approximately 40 bacteria or more. The following reading of a slide offers an illustration. Percentages are obtained by multiplying the number of negative, positive, or filled cells by four:

⊕ + − ⊕ −
− ⊕ ⊕ + ⊕
⊕ ⊕ ⊕ + ⊕
⊕ + ⊕ + −
− − − + ⊕

−28 percent; +72 percent, of which 48 percent are filled (⊕)

The interpretation of the readings was made according to the accompanying chart.

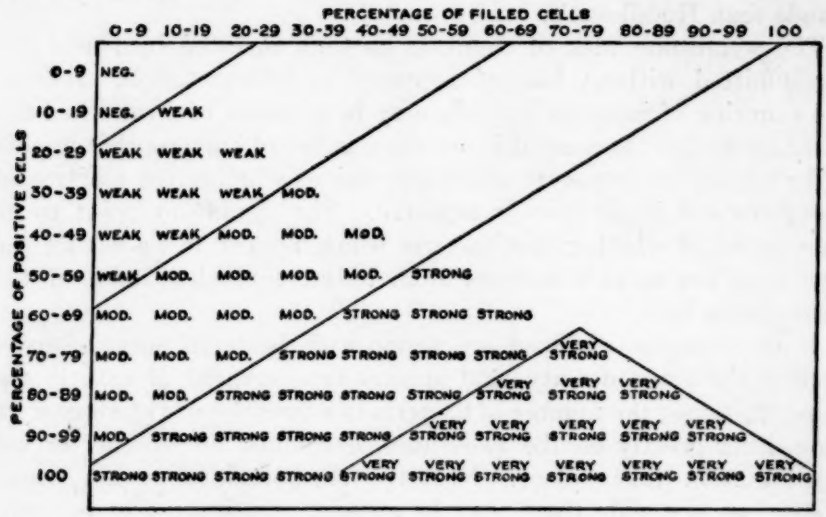


CHART 1.—The interpretation of opsono-cytophagic reactions.

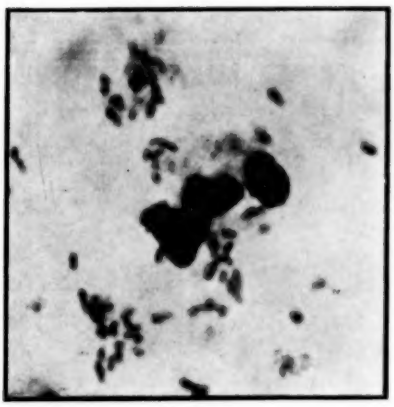
According to this system of interpretation, the total percentage of reacting leucocytes receives a rating on the vertical scale, and the rating is increased on the horizontal scale, according to the percentage of filled cells. There are 5 degrees in the rating of a reaction—negative, weak, moderate, strong, and very strong. Thus, a reading of 72 percent positive cells with 24 percent of them filled would be interpreted as a moderately positive reaction; with 48 percent of cells filled the reaction would be interpreted as strong; with all of the reacting leucocytes filled the reaction would be interpreted as very strong. Photographs of leucocytes which would be read as negative, positive, or "filled" are shown on plate I.

In a general way, our system of readings may be compared with Huddleson's as follows: We would record with a plus sign leucocytes which he would record as slightly or moderately positive. We would record with an encircled plus sign the leucocytes which he would record as markedly positive.

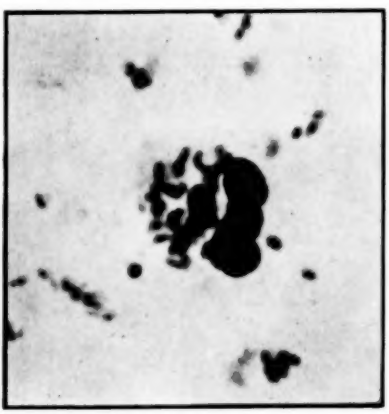
In the consideration of only 25 leucocytes on a slide we follow Huddleson's practice. We believe that other inaccuracies inherent in the technique would obviate further refinement of the readings by the examination of a larger number of cells.

In spite of the various sources of error with which the investigator must contend in preparing and reading the slides, the final result leading to an interpretation of negative, weak, moderate, strong, or very strong phagocytosis may be obtained in repeated tests with considerable accuracy.

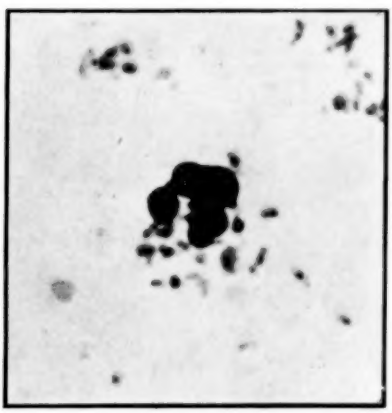
Negative



Negative

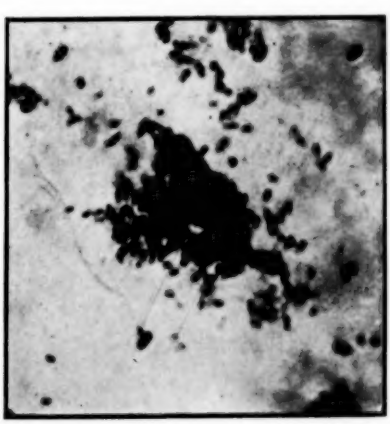


Positive



Positive

Filled



Filled

One of our field workers submitted from several cases repeated smears with different numbers. In some of these cases the reaction was positive, in some it was negative. The results were always consistently positive or negative, though the degree of a positive reaction sometimes varied, as, for example, from a weak to a moderate reaction.

Further evidence of the consistency of results in the opsonocytophagic test was obtained by repeating the test on four different occasions on a volunteer who had an acute attack of brucellosis in 1923. The reactions were as follows:

October 28, 1936, strong.
January 7, 1937, strong.
February 27, 1937, moderate.
July 10, 1937, moderate.

The discrepancy in the readings might have been due either to a change in the immunity status of the subject or to experimental error.

When the test is carried out with normal serum a few leucocytes often appear to have engulfed bacteria. Hence, it would be erroneous to interpret such a test as positive. The line of division between a reading regarded as negative or weakly positive as given on the chart is necessarily arbitrary, as is the line of division between the higher degrees of phagocytosis.

The writer tested the accuracy of her own readings by reexamining 100 slides received from the field investigators. The slides to be reread were first chosen from the records of the previous readings. They were taken in consecutive order, excepting that several series of slides with negative readings were omitted, so that a goodly percentage of positive readings would be included in the 100 slides.

Readings were then made without knowing the previous rating. On 82 percent of the slides the second reading agreed with the first. On 15 percent of the slides there was one degree of error, as, for example, a slide was interpreted as weakly positive in one reading and moderately positive in the other. A third reading confirmed either the one or the other previous reading. On 3 percent of the slides, all of which were unsatisfactory, there was greater error.

From the above results it may be concluded that good slides may be read with considerable accuracy; and that it is useless to attempt to make readings on unsatisfactory slides.

Foshay and LeBlanc have recently published a nomogram for the conversion of inclusion counts into phagocytic index numbers. It came to the writer's attention after the chart for the interpretation of phagocytic readings had been in use for some time.

Dr. Foshay generously responded to the suggestion that the two systems should be correlated. Accordingly, he determined the phagocytic index number on 100 slides prepared by our field investigators which had been previously read and interpreted by the writer. The

slides were selected to include approximately equal numbers with readings of negative, weak, moderate, strong, and very strong reactions. The results give the following correlation between the two systems of interpretation:

Evans' interpretation	Foshay's phagocytic index number
Negative	1-20
Weak	21-30
Moderate	31-60
Strong	61-80
Very strong	81-100

THE INTRADERMAL TEST

Huddleson's *Brucella* nucleo-protein solution, designated "brucellergin", prepared in his laboratory, was used for the allergic reaction. The technique for the preparation of this ether-washed antigen is described in *Brucella Infections in Animals and Man*. The test was made by injecting 0.1 cc of nucleo-protein solution in the lateral surface of the forearm.

Each lot of brucellergin already standardized as to total nitrogen content was standardized for potency on a hypersensitive subject (always the same subject). In the case of each of three lots tested with the 1 to 10 dilution of the material as received from Huddleson, a moderate to severe reaction resulted. In the case of each of two lots tested with the 1 to 100 dilution, a moderate reaction resulted, with an area of erythema of about 5 cm in diameter, and an indurated central area of about 1 cm in diameter.

Five volunteers with histories of having suffered with brucellosis were tested, four with a 1 to 10 and one with a 1 to 100 dilution of brucellergin. In two receiving the 1:10 dilution a general systemic reaction resembling the symptoms of brucellosis was severe enough to incapacitate the subjects for one or two days. The one receiving the 1:100 dilution suffered a slight systemic reaction.

From these results it appeared that it might be unwise to use undiluted material in a patient without having first tested him for hypersensitivity with diluted material. Each field investigator followed his own judgment as to whether he should use undiluted brucellergin in the first test. In subsequent reports the strength of brucellergin used in the several areas will be specified.

Readings were made after 24 and 48 hours in order to exclude early nonspecific reactions. The local reactions varied from weak, with an area of circumscribed erythema and slight edema of about 1.5 centimeters in diameter, to very strong, covering an area of 60 or more square centimeters. In hypersensitive subjects the area tended to lengthen, following the lymphatics, and axial glands were sometimes

swollen. Specific local reactions subsided slowly, leaving a darkened area one or two centimeters in diameter, which often persisted for months.

Goldstein and also Heathman found that the intradermal test with a heat-killed *Brucella* suspension stimulates the production of agglutinins in a large percentage of subjects. Goldstein reported, however, that when a fat-free antigen was used, there was no rise in agglutinin titer in the majority of cases. The following experiment shows that when Huddleson's nucleo-protein is used, it is important that the intradermal test be performed after the sample of blood for serological tests has been taken, because the reaction may stimulate the production of opsonins as well as agglutinins.

TABLE 1.—*The influence of the intradermal test on subsequent serological tests*

Subject	Serological reactions of the first sample		Intradermal reaction	Serological reactions of the second sample	
	Agglutinin titer	Opsono-cytophagic reaction		Agglutinin titer	Opsono-cytophagic reaction
L. B.	[1](1:10)	Negative	−	(1:40)	Weak
C. B.	1:20	Negative	−	1:160	Negative
E. C.	(1:20)	Moderate	+	(1:10)	Negative
L. D.	(1:10)	Negative	−	0	Negative
M. H.	0	Negative	−	0	Negative
M. J.	1:10	Negative	−	1:80	Strong
M. M.	(1:20)	Weak	++++	1:40	Weak
T. P.	0	Weak	++++	1:320	Moderate
R. R.	0	Negative	−	1:160	Negative
G. S.	(1:20)	Negative	−	1:40	Weak
S. S.	1:20	Negative	−	1:80	Moderate
W. W.	1:10	Weak	++++	(1:40)	Negative

[1] Parentheses around the titer indicates that definite agglutination did not occur, but that there was partial sedimentation in the indicated and lower dilutions.

Samples of blood were taken from 12 adult volunteers, then each one was injected with Huddleson's nucleo-protein, diluted 1 to 10. Two weeks later, samples of blood were taken again. Tests for agglutination and opsono-cytophagic reactions were carried out on both samples of blood from each subject, with results as summarized in table 1. There was a development of opsonins in 5 of the 12 subjects, and there was definite development of agglutinins in 7 of the subjects, with a rise from 0 to a titer of 1 to 320 in one case.

REFERENCES

Evans, Alice C.: (1937). Studies on chronic brucellosis. I. Introduction' Pub. Health Rep., **52**: 1072–1077.

Foshay, Lee, and Le Blanc, T. J.: (1937) The derivation of an index number for the opsono-cytophagic test. J. Lab. and Clin. Med., **22**: 1297–1300.

Francis, Edward: Culture medium for Bacterium tularense. (This leaflet may be obtained from the National Institute of Health, Washington, D. C.)

Goldstein, Jacob D.: (1934) Cutaneous reactions in the diagnosis of undulant fever. J. Clin. Invest., **13**: 209–218.

Heathman, Lucy S.: (1934) A survey of workers in packing plants for evidence of Brucella infection. J. Inf. Dis., **55**: 243–265.

Huddleson, I. Forest: (1934) Brucella Infections in Animals and Man. The Commonwealth Fund, New York.

DEATHS DURING WEEK ENDED SEPTEMBER 18, 1937

[From the Weekly Health Index, issued by the Bureau of the Census, Department of Commerce]

	Week ended Sept. 18, 1937	Corresponding week, 1936
Data from 86 large cities in the United States:		
Total deaths	6,816	7,142
Average for 3 prior years	6,988	
Total deaths, first 37 weeks of year	323,179	323,944
Deaths under 1 year of age	483	512
Average for 3 prior years	489	
Deaths under 1 year of age, first 37 weeks of year	20,841	20,580
Data from industrial insurance companies:		
Policies in force	69,840,308	68,465,466
Number of death claims	10,349	11,391
Death claims per 1,000 policies in force, annual rate	7.7	8.7
Death claims per 1,000 policies, first 37 weeks of year, annual rate	10.0	10.1

PREVALENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

CURRENT WEEKLY STATE REPORTS

These reports are preliminary, and the figures are subject to change when later returns are received by the State health officers.

In these and the following tables a zero (0) is to be interpreted to mean that no cases or deaths occurred, while leaders (------) indicate that cases or deaths may have occurred although none was reported.

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended Sept. 25, 1937, and Sept. 26, 1936

Division and State	Diphtheria		Influenza		Measles		Meningococcus meningitis	
	Week ended Sept. 25, 1937	Week ended Sept. 26, 1936	Week ended Sept. 25, 1937	Week ended Sept. 26, 1936	Week ended Sept. 25, 1937	Week ended Sept. 26, 1936	Week ended Sept. 25, 1937	Week ended Sept. 26, 1936
New England States:								
Maine	1	1	------	1	9	32	0	1
New Hampshire	------	------	------	------	1	5	0	0
Vermont	1	------	------	------	3	6	0	0
Massachusetts	2	8	------	------	11	25	1	1
Rhode Island	------	------	------	------	------	6	0	0
Connecticut	5	------	5	------	------	4	1	1
Middle Atlantic States:								
New York	13	26	[1]11	[1]11	43	39	2	6
New Jersey	13	5	5	5	20	16	2	0
Pennsylvania	29	28	------	------	142	30	5	3
East North Central States:								
Ohio	28	23	2	3	54	20	3	3
Indiana	13	10	12	10	6	2	1	1
Illinois	48	14	12	3	43	12	4	5
Michigan	22	13	3	------	18	10	3	1
Wisconsin	5	2	16	12	27	14	2	1
West North Central States:								
Minnesota	3	8	------	1	3	10	4	1
Iowa	3	2	1	------	1	2	0	1
Missouri	14	3	26	58	18	1	0	1
North Dakota	1	------	5	16	1	2	0	0
South Dakota	1	1	------	------	------	2	0	0
Nebraska	3	3	------	------	3	1	0	0
Kansas	3	7	1	------	14	2	1	0
South Atlantic States:								
Delaware	------	------	------	------	------	3	0	0
Maryland [2]	17	13	6	3	4	6	1	3
District of Columbia	4	14	------	------	1	4	0	0
Virginia	41	30	------	------	5	5	1	6
West Virginia	27	14	24	2	25	2	2	2
North Carolina [2]	110	81	1	5	41	4	1	1
South Carolina	21	23	135	130	2	------	1	0
Georgia [2]	30	47	------	------	------	------	0	2
Florida [2]	12	8	------	2	------	1	1	3
East South Central States:								
Kentucky	25	14	10	1	33	9	3	6
Tennessee [4]	37	43	17	11	19	2	2	1
Alabama [2]	43	48	9	3	1	------	2	2
Mississippi [2 3]	30	22	------	------	------	------	1	1

See footnotes at end of table.

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended Sept. 25, 1937, and Sept. 26, 1936—Continued

Division and State	Diphtheria		Influenza		Measles		Meningococcus meningitis	
	Week ended Sept. 25, 1937	Week ended Sept. 26, 1936	Week ended Sept. 25, 1937	Week ended Sept. 26, 1936	Week ended Sept. 25, 1937	Week ended Sept. 26, 1936	Week ended Sept. 25, 1937	Week ended Sept. 26, 1936
West South Central States:								
Arkansas	25	4	8	3	2	----	0	0
Louisiana [3]	13	16	3	3	1	1	0	0
Oklahoma [4]	5	4	17	8	1	----	1	0
Texas [3]	46	31	99	19	42	----	4	0
Mountain States:								
Montana	3	2	----	8	3	----	0	1
Idaho	2	----	----	2	2	----	0	0
Wyoming	----	----	----	----	2	2	0	0
Colorado	7	3	----	----	7	2	1	5
New Mexico	2	1	1	1	6	20	0	0
Arizona	7	2	10	9	1	8	0	0
Utah [3 4]	10	----	2	----	8	1	0	0
Pacific States:								
Washington	1	2	2	1	10	7	0	0
Oregon	1	1	11	13	4	2	1	2
California	33	22	17	29	27	32	6	1
Total	760	599	471	373	664	352	57	62
First 38 weeks of year	16, 195	17, 073	276, 296	141, 416	244, 478	269, 012	4, 446	6, 172

Division and State	Poliomyelitis		Scarlet fever		Smallpox		Typhoid and paratyphoid fevers	
	Week ended Sept. 25, 1937	Week ended Sept. 26, 1936	Week ended Sept. 25, 1937	Week ended Sept. 26, 1936	Week ended Sept. 25, 1937	Week ended Sept. 26, 1936	Week ended Sept. 25, 1937	Week ended Sept. 26, 1936
New England States:								
Maine	6	6	5	7	0	0	1	2
New Hampshire	1	0	----	6	0	0	0	0
Vermont	3	0	1	4	0	0	0	0
Massachusetts	21	1	51	50	0	0	8	3
Rhode Island	2	0	5	13	0	0	0	0
Connecticut	11	3	17	10	0	0	2	1
Middle Atlantic States:								
New York	61	16	99	130	0	0	20	25
New Jersey	21	2	26	16	0	0	11	16
Pennsylvania	66	1	125	110	0	0	51	22
East North Central States:								
Ohio	28	27	111	99	0	0	27	30
Indiana	10	7	60	60	0	0	5	8
Illinois	66	75	156	107	0	5	22	29
Michigan	63	9	144	88	2	1	12	11
Wisconsin	44	4	46	76	0	0	4	2
West North Central States:								
Minnesota	53	3	32	19	3	0	8	3
Iowa	31	7	36	12	0	2	7	2
Missouri	33	2	75	12	0	0	22	17
North Dakota	1	2	5	7	6	4	2	2
South Dakota	6	1	6	6	0	0	2	2
Nebraska	7	3	6	14	2	0	1	1
Kansas	36	4	64	31	0	0	7	5
South Atlantic States:								
Delaware	1	0	----	----	0	0	1	1
Maryland [3]	9	5	19	12	0	0	18	16
District of Columbia	6	1	7	4	0	0	0	2
Virginia	4	3	21	15	0	0	22	26
West Virginia	3	4	47	30	0	0	10	24
North Carolina [3]	1	1	54	45	0	0	18	30
South Carolina	0	0	9	8	0	0	14	20
Georgia	1	11	34	15	0	0	15	21
Florida [3]	2	0	3	2	0	0	4	5

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended Sept. 25, 1937, and Sept. 26, 1936—Continued

Division and State	Poliomyelitis		Scarlet fever		Smallpox		Typhoid and paratyphoid fevers	
	Week ended Sept. 25, 1937	Week ended Sept. 26, 1936	Week ended Sept. 25, 1937	Week ended Sept. 26, 1936	Week ended Sept. 25, 1937	Week ended Sept. 26, 1936	Week ended Sept. 25, 1937	Week ended Sept. 26, 1936
East South Central States:								
Kentucky	5	1	58	27	0	1	38	26
Tennessee [4]	1	23	33	27	0	0	14	35
Alabama [5]	1	6	17	13	0	0	7	32
Mississippi [2] [3]	9	3	7	15	0	0	6	17
West South Central States:								
Arkansas	12	1	9	6	0	0	22	10
Louisiana [5]	5	1	10	1	0	0	26	23
Oklahoma [5]	13	0	15	10	1	0	12	12
Texas [3]	17	2	23	15	0	0	64	24
Mountain States:								
Montana	4	1	27	29	5	4	1	10
Idaho	2	0	12	9	1	0	0	4
Wyoming	8	0	9	4	0	0	0	1
Colorado	9	9	10	15	0	0	27	10
New Mexico	1	4	2	6	0	0	15	23
Arizona	0	0	4	5	0	0	2	0
Utah [2] [4]	2	0	81	7	0	0	1	0
Pacific States:								
Washington	6	5	16	19	25	3	6	4
Oregon	3	5	7	15	1	0	2	2
California	35	18	67	93	3	0	21	13
Total	730	277	1,671	1,324	49	20	578	572
First 38 weeks of year	7,121	2,538	170,459	183,975	8,233	6,090	11,192	10,342

[1] New York City only.
[2] Week ended earlier than Saturday.
[3] Typhus fever, week ended Sept. 25, 1937, 60 cases, as follows: North Carolina, 1; Georgia, 24; Florida, 7; Alabama, 14; Mississippi, 1; Louisiana, 3; Texas, 10.
[4] Rocky Mountain spotted fever, week ended Sept. 25, 1937, 3 cases, as follows: Tennessee, 1; Utah, 2.
[5] Figures for 1936 are exclusive of Oklahoma City and Tulsa.

SUMMARY OF MONTHLY REPORTS FROM STATES

The following summary of cases reported monthly by States is published weekly and covers only those States from which reports are received during the current week:

State	Meningococcus meningitis	Diphtheria	Influenza	Malaria	Measles	Pellagra	Poliomyelitis	Scarlet fever	Smallpox	Typhoid fever
August 1937										
Florida	2	23	4	169	26	1	3	7	0	5
Kansas	4	10	2	10	20		56	91		30
Louisiana	4	48	37	132	4	7	25	26	0	81
Maine					10		35	11	0	12
Maryland	7	16	3	3	31	1	29	31	0	56
Massachusetts	11	17		6	107		147	112	0	12
Mississippi	2	50	642	7,951	217	289	39	19	0	47
Montana	1	7	1		30		11	49	57	17
New Mexico	2	15		6	91	3	4	9	0	39
New York	27	55		16	525		160	323	11	92
North Dakota	1	12	6		2		0	25	12	6
Oklahoma	11	18	42	312	19	18	104	30	0	111
Rhode Island	1	1			7		4	16	0	3
South Dakota	4	1	3		3		6	32	7	
Texas	15	99	174	2,525	162	177	182	110	0	337

Summary of monthly reports from States—Continued

August 1937

	Cases
Anthrax:	
Louisiana	1
Mississippi	2
North Dakota	1
South Dakota	1
Texas	1
Chicken pox:	
Florida	5
Kansas	8
Louisiana	1
Maine	23
Maryland	12
Massachusetts	105
Mississippi	147
Montana	39
New Mexico	6
New York	257
North Dakota	8
Oklahoma	7
Rhode Island	5
South Dakota	12
Texas	40
Conjunctivitis:	
New Mexico	9
Oklahoma	1
Dengue:	
Florida	1
Mississippi	2
Texas	9
Diarrhea:	
Maryland	81
Dysentery:	
Kansas (amoebic)	1
Kansas (bacillary)	3
Louisiana (amoebic)	7
Louisiana (bacillary)	2
Maryland (bacillary)	80
Massachusetts (bacillary)	20
Mississippi (amoebic)	126
Mississippi (bacillary)	820
New Mexico (amoebic)	2
New Mexico (bacillary)	9
New Mexico (unspecified)	10
New York (amoebic)	8
New York (bacillary)	114
Oklahoma	18
Texas (amoebic)	5
Texas (bacillary)	263
Encephalitis, epidemic or lethargic:	
Kansas	8
Louisiana	4
Maryland	1
Massachusetts	2
Montana	1
New York	3
North Dakota	1
Oklahoma	5
South Dakota	4
Texas	4
Food poisoning:	
New Mexico	1
German measles:	
Kansas	5
Maine	3
Maryland	1
Massachusetts	26
Montana	6
New York	87
Rhode Island	3
Hookworm disease:	
Florida	599
Louisiana	89
Mississippi	804
Impetigo contagiosa:	
Kansas	5
Maryland	24
Montana	2
Lead poisoning:	
Massachusetts	1
Leprosy:	
Louisiana	2
Mumps:	
Florida	29
Kansas	69
Louisiana	1
Maine	45
Maryland	15
Massachusetts	95
Mississippi	149
Montana	49
New Mexico	4
Oklahoma	3
Rhode Island	4
South Dakota	8
Texas	106
Ophthalmia neonatorum:	
Florida	1
Louisiana	2
Maryland	1
Massachusetts	83
Mississippi	6
New Mexico	1
New York [1]	7
Paratyphoid fever:	
Florida	1
Kansas	1
Maine	1
Maryland	2
Massachusetts	34
Montana	3
New Mexico	1
New York	18
Texas	26
Psittacosis:	
New York (delayed report)	3
Puerperal septicemia:	
Mississippi	39
New Mexico	5
Rabies in animals:	
Louisiana	14
Massachusetts	13
Mississippi	13
New York [1]	9
Rabies in man:	
Florida	1
Louisiana	1
Mississippi	2
Rocky Mountain spotted fever:	
Maryland	5
Montana	3
New York	1
Rhode Island (imported)	2
Septic sore throat:	
Kansas	2
Louisiana	9
Maine	3
Maryland	4
Massachusetts	7
Septic sore throat—Con.	
Montana	10
New Mexico	1
New York	30
Oklahoma	34
Rhode Island	2
South Dakota	5
Tetanus:	
Louisiana	4
Maine	1
New York	7
Oklahoma	1
Trachoma:	
Massachusetts	3
Mississippi	10
Montana (delayed report)	93
New Mexico	2
Oklahoma	8
Trichinosis:	
Massachusetts	3
New York	2
Tularaemia:	
Florida	1
Louisiana	2
New Mexico	1
Texas	2
Typhus fever:	
Florida	12
Louisiana	6
Mississippi	4
New York	1
Texas	64
Undulant fever:	
Florida	4
Kansas	8
Louisiana	5
Maine	1
Maryland	6
Massachusetts	3
Mississippi	3
Montana	1
New Mexico	4
New York	18
Oklahoma	24
Rhode Island	2
South Dakota	1
Texas	7
Vincent's infection:	
Florida	31
Kansas	16
Maryland	15
New York [1]	68
Oklahoma	7
Whooping cough:	
Florida	23
Kansas	282
Louisiana	63
Maine	93
Maryland	418
Massachusetts	682
Mississippi	509
Montana	185
New Mexico	93
New York	1,610
North Dakota	180
Oklahoma	52
Rhode Island	112
South Dakota	53
Texas	628

[1] Exclusive of New York City.

PLAGUE INFECTION IN CALIFORNIA

Dr. W. M. Dickie, director of public health of California, under date of September 22, 1937, states that plague infection has been demonstrated in pools of fleas taken from rodents and in tissue specimens from rodents in California as follows:

San Bernardino County.—160 fleas from 20 golden mantled squirrels, 30 fleas from 18 *fisheri* squirrels, 70 fleas from 13 *fisheri* squirrels, 56 fleas from 27 golden mantled squirrels, collected on August 12; 98 fleas from 21 *fisheri* squirrels collected August 20; 140 fleas from 11 *fisheri* squirrels, 30 fleas from 36 golden mantled squirrels collected August 23; 253 fleas from 11 *fisheri* squirrels collected August 27; 213 fleas from 33 *fisheri* squirrels collected September 8.

San Mateo County.—16 fleas from 1 *beecheyi* squirrel and 46 fleas from 1 *beecheyi* squirrel collected September 22, 1936.

El Dorado County, Lake Tahoe region.—16 fleas from 27 golden mantled squirrels collected August 31.

Fresno County.—Specimens of tissue from 3 *beecheyi* squirrels received at the laboratory September 16 were proved positive for plague, by animal inoculation, on September 20.

WEEKLY REPORTS FROM CITIES

City reports for week ended Sept. 18, 1937

This table summarizes the reports received weekly from a selected list of 140 cities for the purpose of showing a cross section of the current urban incidence of the communicable diseases listed in the table. Weekly reports are received from about 700 cities, from which the data are tabulated and filed for reference.

State and city	Diphtheria cases	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
		Cases	Deaths								
Data for 90 cities:											
5-year average	160	76	16	107	304	377	4	346	96	908	------
Current week [1]	83	38	12	162	263	330	2	325	84	1,044	------
Maine:											
Portland	0	------	0	0	0	0	0	0	0	14	29
New Hampshire:											
Concord	0	------	0	0	0	0	0	0	0	0	1
Manchester	0	------	0	0	1	0	0	0	0	0	14
Nashua	0	------	0	0	0	0	0	1	0	0	9
Vermont:											
Barre	0	------	0	1	0	0	0	0	0	0	2
Burlington	0	------	0	0	0	0	0	0	2	2	6
Rutland	0	------	0	0	0	0	0	0	0	0	1
Massachusetts:											
Boston	0	------	0	3	8	14	0	2	0	23	182
Fall River	0	------	0	0	2	1	0	0	0	23	20
Springfield	0	------	0	0	0	1	0	1	1	4	32
Worcester	0	------	0	1	2	3	0	1	0	3	36
Rhode Island:											
Providence	0	------	0	2	2	3	0	3	0	33	53
Connecticut:											
Bridgeport	0	1	1	0	0	0	0	0	0	0	25
Hartford	0	------	0	0	2	5	0	0	2	1	23
New Haven	0	------	0	2	1	2	0	0	0	2	34
New York:											
Buffalo	0	------	0	1	3	3	0	11	1	9	121
New York	11	6	1	8	45	25	0	75	20	127	1,117
Rochester	0	------	1	1	1	1	0	0	2	6	46
Syracuse	0	------	0	7	1	1	0	2	0	25	46
New Jersey:											
Camden	5	------	0	0	1	0	0	0	0	0	25
Newark	0	------	0	2	2	2	0	5	0	14	70
Trenton	0	------	0	3	0	1	0	3	1	7	14
Pennsylvania:											
Philadelphia	3	------	0	2	14	12	0	18	8	24	293
Pittsburgh	1	------	3	31	10	12	0	8	3	24	118
Reading	0	------	0	0	1	0	0	0	0	0	9
Scranton	2	------	------	0	------	1	0	------	0	5	------

[1] Figures for Columbus and Boise estimated; reports not received.

City reports for week ended Sept. 18, 1937—Continued

State and city	Diphtheria cases	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
		Cases	Deaths								
Ohio:											
Cincinnati	0	1	0	0	2	4	0	10	1	20	113
Cleveland	3	4	0	6	4	14	0	10	4	35	159
Toledo	0	----	0	3	2	4	0	4	2	17	49
Indiana:											
Anderson	0	----	0	1	1	4	0	0	0	5	12
Fort Wayne	0	----	0	0	1	1	0	0	0	0	24
Indianapolis	0	----	0	1	5	5	0	3	0	17	78
South Bend	0	----	0	0	1	0	0	0	0	1	10
Terre Haute	1	----	0	0	0	3	0	0	1	0	16
Illinois:											
Alton	0	----	0	0	0	1	0	0	0	0	8
Chicago	5	2	2	19	19	41	0	34	2	62	536
Elgin	0	----	0	0	0	0	0	0	0	0	3
Moline	0	----	0	0	0	3	0	0	0	2	5
Springfield	0	----	0	0	1	1	0	0	0	4	18
Michigan:											
Detroit	5	3	0	13	9	30	0	23	2	84	246
Flint	0	----	0	0	1	5	0	0	1	2	20
Grand Rapids	0	----	0	0	1	2	0	1	1	13	25
Wisconsin:											
Kenosha	0	----	0	0	1	2	0	0	0	2	11
Madison	1	----	0	0	0	1	0	0	0	19	19
Milwaukee	0	1	1	16	1	3	0	2	0	44	74
Racine	0	----	0	1	0	2	0	0	0	2	9
Superior	0	----	0	0	0	0	0	0	0	0	8
Minnesota:											
Duluth	0	----	0	0	1	2	0	0	0	5	13
Minneapolis	0	----	0	2	5	6	0	1	1	18	72
St. Paul	0	----	0	2	1	3	1	1	0	17	54
Iowa:											
Cedar Rapids	0	----	----	0	----	0	0	----	0	0	----
Des Moines	0	----	0	0	0	5	0	0	0	3	20
Sioux City	0	----	----	0	----	1	0	----	0	2	----
Waterloo	1	----	----	0	----	3	0	----	0	0	----
Missouri:											
Kansas City	1	----	0	2	3	2	0	0	2	2	79
St. Joseph	0	----	0	0	2	1	0	0	0	0	23
St. Louis	4	----	1	9	6	19	0	6	1	15	222
North Dakota:											
Fargo	1	----	0	0	1	1	0	0	0	28	6
Grand Forks	0	----	----	0	----	0	0	----	0	5	----
Minot	0	----	0	0	0	0	0	0	0	3	4
South Dakota:											
Aberdeen	0	----	----	0	----	0	0	----	0	4	----
Sioux Falls	0	----	0	0	0	0	0	0	0	0	9
Nebraska:											
Omaha	0	----	0	0	2	1	0	3	0	0	46
Kansas:											
Lawrence	0	----	0	0	0	0	0	0	0	4	2
Topeka	0	----	0	0	1	0	0	0	1	7	8
Wichita	0	----	0	0	4	0	0	0	0	4	24
Delaware:											
Wilmington	0	----	0	0	1	1	0	1	0	0	18
Maryland:											
Baltimore	2	3	0	2	10	6	0	10	2	57	197
Cumberland	0	----	0	0	0	2	0	0	0	1	18
Frederick	0	----	0	0	0	0	0	0	0	0	3
Dist. of Columbia:											
Washington	2	----	0	0	6	5	0	9	1	2	140
Virginia:											
Lynchburg	5	----	0	0	1	0	0	0	0	1	12
Norfolk	0	----	0	0	4	0	0	1	0	0	30
Richmond	0	----	0	0	3	3	0	1	1	5	49
Roanoke	2	----	0	0	0	0	0	0	0	11	15
West Virginia:											
Charleston	0	----	0	0	1	3	0	0	0	0	15
Huntington	1	----	0	0	----	0	0	----	0	0	----
Wheeling	0	----	0	0	2	0	0	0	0	8	23
North Carolina:											
Gastonia	0	----	----	0	----	1	0	----	0	5	----
Raleigh	0	----	0	0	1	0	0	2	0	7	9
Wilmington	0	----	0	0	0	0	0	0	0	1	8
Winston-Salem	0	----	0	0	0	1	0	0	0	2	16
South Carolina:											
Charleston	0	1	0	0	2	1	0	2	3	0	22
Columbia	0	----	0	0	0	0	0	0	0	0	5
Greenville	0	----	0	0	1	0	0	0	0	1	22

City reports for week ended Sept. 18, 1937—Continued

State and city	Diphtheria cases	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
		Cases	Deaths								
Georgia:											
Atlanta	2		0	0	3	4	0	4	1	11	64
Brunswick	0		0	0	0	0	0	0	0	2	5
Savannah	1	3	0	0	2	0	0	4	0	0	34
Florida:											
Miami	3		0	2	2	0	0	5	0	0	28
Tampa	0	1	1	2	0	0	0	0	0	0	16
Kentucky:											
Ashland	0		0	0	0	0	0	2	0	0	16
Covington	1		0	0	0	0	0	1	0	9	6
Lexington	0		0	0	1	1	0	2	0	5	21
Louisville	1		0	2	2	11	0	3	0	26	44
Tennessee:											
Knoxville	4	1	0	0	1	0	0	0	1	0	18
Memphis	2		0	0	2	1	0	0	3	13	75
Nashville	0		0	0	0	2	0	0	1	13	32
Alabama:											
Birmingham	1	1	0	2	3	3	0	5	0	4	60
Mobile	1		0	0	0	0	0	1	0	0	19
Montgomery	1			0		1	0		0	0	
Arkansas:											
Fort Smith	0			0		1	0		0	5	
Little Rock	1		0	0	5	0	0	1	0	0	6
Louisiana:											
Lake Charles	0			0		0	0		0	0	
New Orleans	2	1	0	0	9	3	0	9	1	5	120
Shreveport	0		0	0	4	5	0	2	0	0	36
Oklahoma:											
Muskogee	0		0	0	0	0	0	0	0	0	
Oklahoma City	0		0	0	2	4	0	1	1	0	38
Tulsa	1			0		0	0		0	3	
Texas:											
Dallas	3		0	0	2	1	0	2	5	8	51
Fort Worth	4		0	0	2	2	0	0	0	8	39
Galveston	0		0	0	0	0	0	0	0	0	11
Houston	7		0	3	6	1	0	3	2	9	74
San Antonio	1		0	0	3	0	0	6	1	2	48
Montana:											
Billings	0		0	0	0	1	0	0	0	0	14
Great Falls	0		0	0	0	1	0	0	0	5	5
Helena	0		0	0	0	0	0	0	0	3	6
Missoula	0		0	0	1	1	0	2	0	0	11
Idaho:											
Boise											
Colorado:											
Colorado Springs	0		0	0	0	0	0	0	0	1	10
Denver	5		0	6	3	7	1	3	3	12	76
Pueblo	0		0	0	0	0	0	0	0	0	9
Utah:											
Salt Lake City	1		0	0	2	9	0	0	0	4	27
Washington:											
Seattle	0		0	3	3	1	0	2	2	28	91
Spokane	0		0	3	1	5	0	1	0	4	30
Tacoma	0		0	0	1	1	0	0	0	5	26
Oregon:											
Portland	2		0	2	1	4	1	3	0	0	68
Salem	0			0		0	0		0	0	
California:											
Los Angeles	4	6	0	3	11	16	0	16	2	47	363
Sacramento	0		0	1	0	2	0	3	0	7	15
San Francisco	0	1	0	1	5	6	0	12	0	42	193

City reports for week ended Sept. 18, 1937—Continued

State and city	Meningococcus meningitis		Poliomyelitis cases
	Cases	Deaths	
Maine:			
Portland	0	0	2
Massachusetts:			
Boston	0	0	24
Springfield	0	0	2
Worcester	0	0	2
Rhode Island			
Providence	0	0	3
Connecticut:			
Bridgeport	0	0	1
Hartford	0	0	2
New Haven	0	0	1
New York:			
Buffalo	1	0	7
New York	3	1	33
Rochester	0	0	1
Syracuse	0	0	5
New Jersey			
Camden	0	0	3
Newark	1	0	6
Pennsylvania:			
Philadelphia	2	0	14
Reading	0	0	1
Ohio:			
Cincinnati	0	0	4
Cleveland	0	0	11
Toledo	0	0	2
Indiana:			
Indianapolis	0	0	3
South Bend	0	0	1
Terre Haute	0	0	1
Illinois:			
Alton	1	1	0
Chicago	0	0	36
Elgin	0	0	1
Springfield	0	0	3
Michigan:			
Detroit	0	0	24
Flint	0	0	1
Wisconsin:			
Madison	0	0	3
Milwaukee	0	0	14
Racine	1	0	2
Minnesota:			
Duluth	0	0	2
Minneapolis	0	0	16
St. Paul	0	0	23
Iowa:			
Des Moines	0	0	4
Sioux City	0	0	1
Missouri:			
Kansas City	0	0	13
St. Louis	0	0	6
Nebraska:			
Omaha	0	0	13
Kansas:			
Topeka	0	0	1
Wichita	0	0	2
Delaware:			
Wilmington	0	0	2
Maryland:			
Baltimore	0	0	1
District of Columbia:			
Washington	0	0	2
Virginia:			
Norfolk	1	0	0
Richmond	1	1	0
Georgia:			
Brunswick	0	0	3
Florida:			
Miami	0	0	1
Tampa	1	0	0
Kentucky:			
Ashland	1	0	0
Alabama:			
Birmingham	1	1	0
Arkansas:			
Little Rock	0	0	1
Louisiana:			
New Orleans	2	0	1
Shreveport	0	1	0
Oklahoma:			
Tulsa	0	0	3
Texas:			
Dallas	0	0	4
Houston	0	0	5
Colorado:			
Colorado Springs	0	0	1
Pueblo	0	0	4
Utah:			
Salt Lake City	0	0	4
Washington:			
Seattle	0	0	4
Oregon:			
Portland	0	0	1
California:			
Los Angeles	0	0	7
Sacramento	0	0	4
San Francisco	0	0	2

Encephalitis, epidemic or lethargic.—Cases: New York, 6; Cleveland, 2; Alton, 1; St. Louis, 76; Great Falls, 1; Los Angeles, 1; Sacramento, 2; San Francisco, 1.

Pellagra.—Cases: Lynchburg, 8; Atlanta, 1; Savannah, 1; Birmingham, 2; New Orleans, 1; Dallas, 1; San Francisco, 1.

Typhus fever.—Cases: Atlanta, 1; Savannah, 2; New Orleans, 1; Dallas, 1.

FOREIGN AND INSULAR

CANADA

Provinces—Communicable diseases—2 weeks ended September 11, 1937.—During the 2 weeks ended September 11, 1937, cases of certain communicable diseases were reported by the Department of Pensions and National Health of Canada as follows:

Disease	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia	Total
Cerebrospinal meningitis			1		6	1			1	9
Chicken pox				42	51	8	20	15	36	172
Diphtheria		2	2	83	9	1	1		3	101
Dysentery				2	29					31
Erysipelas				6		4	1	4		15
Influenza	2	14		1	6					23
Lethargic encephalitis	1	1			1					3
Measles		9		66	109	6	33	32	56	311
Mumps					31	6	10	1	13	61
Paratyphoid fever					6				5	11
Pneumonia	3	1			15				3	22
Poliomyelitis		7	40	34	782	60	87	39	1	1,050
Scarlet fever		7	6	89	76	24	45	31	28	306
Trachoma						1	1		2	4
Tuberculosis	5	4	22	113	74	1	1	5	32	257
Typhoid fever			16	91	23	2	22	1	4	159
Undulant fever				4	4		2			10
Whooping cough		2		307	236	68	4	13	46	676

Vital statistics—First quarter 1937.—The Bureau of Statistics of the Dominion of Canada has published the following preliminary statistics for the first quarter of 1937. The rates are computed on an annual basis. There were 18.9 live births per 1,000 population during the first quarter of 1937 and 20.3 per 1,000 population for the same quarter of 1936. The death rate was 11.5 per 1,000 population for the first quarter of 1937 and 10.4 per 1,000 population for the first quarter of 1936. The infant mortality rate for the first quarter of 1937 was 87 per 1,000 live births and 69 per 1,000 live births in the corresponding quarter of 1936. The maternal death rate was 5.4 per 1,000 live births for the first quarter of 1937 and 6.2 per 1,000 live births for the same quarter of 1936.

The accompanying tables give the numbers of births, deaths, and marriages by Provinces for the first quarter of 1937, and deaths from certain causes in Canada for the first quarter of 1937 and the corresponding quarter of 1936.

Number of births, deaths, and marriages, first quarter 1937

Province	Live births	Deaths (exclusive of stillbirths)	Deaths under 1 year of age	Maternal deaths	Marriages
Canada [1]	51,623	31,534	4,482	277	12,617
Prince Edward Island	449	283	33	4	93
Nova Scotia	2,469	1,539	231	4	686
New Brunswick	2,582	1,396	272	8	504
Quebec	17,677	9,353	1,848	89	3,024
Ontario	14,924	10,887	1,003	84	4,579
Manitoba	3,178	1,822	274	11	856
Saskatchewan	4,274	1,986	334	29	883
Alberta	3,504	1,794	290	27	1,000
British Columbia	2,566	2,474	197	21	992

Cause of death	Canada [1] (first quarter)		Province, first quarter 1937								
	1936	1937	Prince Edward Island	Nova Scotia	New Brunswick	Quebec	Ontario	Manitoba	Saskatchewan	Alberta	British Columbia
Automobile accidents	147	223	1	17	9	44	120	4	3	4	21
Cancer	2,938	2,887	39	160	98	746	1,066	190	188	154	246
Diarrhea and enteritis	454	388	1	10	8	249	60	20	13	18	9
Diphtheria	65	76		5	1	53	7	6	3	1	
Diseases of the arteries	2,458	2,659	21	119	114	547	1,296	155	115	114	178
Diseases of the heart	4,452	4,617	30	201	164	1,087	1,999	267	245	217	407
Homicides	45	37	1	2	1	10	13	3	2	1	4
Influenza	1,363	3,300	19	121	95	910	1,088	220	268	306	273
Measles	135	290		1	4	71	11	3	101	42	57
Nephritis	1,747	1,813	20	84	56	790	562	51	80	71	99
Pneumonia	2,376	2,796	34	136	156	803	948	190	174	168	187
Poliomyelitis	13	8		1		3	2	1	1		
Puerperal causes	277	345	4	4	8	89	84	11	29	27	21
Scarlet fever	82	83		3	1	38	18	4	4	11	4
Smallpox	2	1								1	
Suicides	229	220	2	7	3	29	98	17	17	22	25
Tuberculosis	1,724	1,719	15	102	95	712	356	124	71	79	165
Typhoid fever and paratyphoid fever	60	43		2	1	29	6	1	1	2	1
Whooping cough	174	192	1	16	3	90	34	3	21	18	6
Violent deaths	907	935	5	59	32	183	376	35	64	63	118

[1] Exclusive of Yukon and the Northwest Territories.

GREAT BRITAIN

England and Wales—Infectious diseases—13 weeks ended July 3, 1937.—During the 13 weeks ended July 3, 1937, certain infectious diseases were reported in England and Wales as follows:

Disease	Cases	Disease	Cases
Diphtheria	12,347	Puerperal pyrexia	1,570
Ophthalmia neonatorum	1,376	Scarlet fever	20,817
Pneumonia	10,591	Typhoid fever	342
Puerperal fever	490		

England and Wales—Vital statistics—Second quarter 1937.—During the quarter ended June 30, 1937, 163,867 live births and 118,524 deaths were registered in England and Wales. The following statistics are taken from the Quarterly Return of Births, Deaths, and Marriages, issued by the Registrar General of England and Wales, and are provisional:

Birth and death rates in England and Wales, quarter ended June 30, 1937

Annual rates per 1,000 population:	
Live births	16.1
Stillbirths	.65
Deaths, all causes	11.6
Deaths under 1 year of age	[1] 54
Deaths from:	
Diarrhea and enteritis (under 2 years of age)	[1] 5.2
Diphtheria	.06
Annual rates per 1,000 population—Continued.	
Deaths from—Continued.	
Influenza	0.12
Measles	.03
Scarlet fever	.01
Typhoid fever and paratyphoid fever	0.0
Violence	.54
Whooping cough	.04

[1] Per 1,000 live births.

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER

NOTE.—A table giving current information of the world prevalence of quarantinable diseases appeared in the PUBLIC HEALTH REPORTS for September 24, 1937, pages 1354–1368. A similar cumulative table will appear in the PUBLIC HEALTH REPORTS to be issued October 29, 1937, and thereafter, at least for the time being, in the issue published on the last Friday of each month.

Cholera

China—Hong Kong.—During the week ended September 18, 1937, 106 cases of cholera with 66 deaths were reported in Hong Kong, China.

Japan—Hiroshima.—During the week ended September 25, 1937, 32 cases of cholera with several deaths were reported in Hiroshima, Japan.

Plague

United States—California.—A report of plague infection in California appears on pages 1432–33 of this issue of PUBLIC HEALTH REPORTS.

Smallpox

Mexico.—During the month of July 1937, smallpox was reported in Mexico as follows: Mexico, D. F., 18 cases, 3 deaths; Monterrey, Nuevo Leon State, 1 case, 1 death; Merida, Yucatan State, 1 case.

Typhus Fever

Egypt—Cairo.—During the week ended September 18, 1937, 1 case of typhus fever was reported in Cairo, Egypt.

Mexico.—During the month of July 1937, typhus fever was reported in Mexico as follows: Guadalajara, Jalisco State, 1 case; Guanajuato, Guanajuato State, 3 cases; Mexico, D. F., 16 cases, 5 deaths; Pachuca, Hidalgo State, 1 death; Toluca, Mexico State, 14 cases.

Yellow Fever

French Equatorial Africa—Ubangi Chari Territory—Bangui.—During the week ended September 18, 1937, 1 death from yellow fever was reported in Bangui, Ubangi Chari Territory, French Equatorial Africa.

Ivory Coast—Touba.—During the week ended September 25, 1937, 1 death from suspected yellow fever was reported in Touba, Ivory Coast.